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Exercise-induced B cell mobilisation: Preliminary evidence for an influx of immature cells into the bloodstream

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Exercise-induced B cell mobilisation: preliminary evidence for an influx of immature cells into the bloodstream

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ABSTRACT

The number of peripheral blood B lymphocytes doubles during acute exercise, but the phenotypic composition of this response remains unknown. In two independent exercise studies, using complimentary phenotyping strategies, we investigated the mobilisation patterns of distinct B cell subsets. In study one, nine healthy males (mean \pm SD age: 22.1 ± 3.4 years) completed a continuous cycling bout at 80% $\dot{V}O_{2\text{MAX}}$ for 20 mins. In study two, seven healthy experienced cyclists (mean \pm SD age: 29.9 ± 4.7 years) completed a 30 min cycling trial at a workload corresponding to +5% of the individual blood lactate threshold. In study one, CD3–CD19+ B cell subsets were classified into immature (CD27–CD10+), naïve (CD27–CD10–), memory (CD27+CD38–), plasma cells / plasmablasts (CD27+CD38+) and finally, recently purported ‘B1’ cells (CD27+CD43+CD69–). In study two, CD20+ B cells were classified into immature (CD27–IgD–), naïve (CD27–IgD+), and IgM+/IgG+/IgA+ memory cells (CD27+IgD–). Total B cells exhibited a mean increase of 88% (study one) and 60% (study two) during exercise. In both studies, immature cells displayed the greatest increase, followed by memory cells, then naïve cells (study one: immature 130% > mature 105% > naïve 84%; study two: immature 110% > mature 56% > naïve 38%). Our findings show that, unlike T cells and NK cells, B cell mobilisation is not driven by effector status, and, for the first time, that B cell mobilisation during exercise is comprised of immature CD27– IgD–/CD10+ cells.

KEY WORDS: B lymphocytes, exercise, homing, immune-surveillance, lymphocytosis

SHORT TITLE: Exercise-induced B cell subset mobilisation

1. INTRODUCTION

Acute exercise causes a profound, rapid and transient change to the cellular composition of blood. Lymphocyte numbers increase markedly, falling below resting levels shortly after exercise cessation, and then returning to pre-exercise levels a few hours later (47). The primary mechanism for

this effect is the expression of β 2-adrenergic receptors on the surface of lymphocytes (10), that, in response to exercise-induced elevations in catecholamine levels, induce lymphocyte detachment from the vascular endothelium and subsequent lymphocyte release into the peripheral bloodstream (2, 34). The most exercise-responsive lymphocyte populations (with the highest β 2-adrenergic receptor expression density) are natural killer (NK) cells and effector memory CD8⁺ T cells (3, 4, 43). These populations are capable of mounting rapid effector functions that may bolster immune competence (3). Like their lymphoid counterparts, B cells also express β 2-adrenergic receptors (26, 39) and show marked mobilisation responses during exercise, for example increasing by 100% in response to an acute (24 min) bout of intensive cycling at 85% W_{max} (4). However, changes to the phenotypic composition of peripheral blood B cells during exercise has not been well characterised, and it remains unknown whether B cells with a capacity to mount rapid effector functions are preferentially mobilised by exercise (i.e., in a parallel fashion to effector T cell and NK cells). Such effects would lend support to the theory that exercise causes a global priming of the immune system whereby leukocytes with an effector phenotype are mobilised into the bloodstream to enable their migration to sites of injury and subsequent antigenic challenge (7, 8).

Prior research using a rodent model of acute stress may lend support to this theory. Indeed, it was purported that class-switched B cells (i.e., memory cells) are more sensitive to stress than their naïve counterparts, and may be redeployed to peripheral sites post-stress to screen for, and facilitate responses to, cognate antigen (8). Using CD62L (L-selectin) as a marker to differentiate naïve B cells (CD62L⁺) from memory B cells (CD62L[−]), it was shown that CD62L[−] B cells trafficked to a greater extent than CD62L⁺ B cells during acute stress (8). Although loss of CD62L on B cells is not an exclusive indicator of memory status or homing capacity to peripheral sites (33, 37), CD62L[−] cells have been shown to predominate skin sites, thus giving some support to the hypothesis that these cells are mobilised to sites of potential injury or infection (17, 49). To date, in-depth phenotyping of B cell subset changes in response to acute exercise or stress has not been conducted. However, it has been shown that some humoral processes with B cell lineage involvement, such as immunoglobulin (20) and free light chain synthesis (23), are impacted by regular exercise in humans. Thus, perturbations in

these parameters, that are likely to be influenced by the phenotype of cells present in the bloodstream or tissues, may have consequences for health via changes to immune competency or reduced low-grade inflammation (23). It is therefore important to characterise how a single bout of exercise impacts B cell sub-type re-distribution in humans.

Thus, in this work we investigated the mobilisation of distinct B cell subsets in two independent acute exercise studies. In both exercise studies we used the cell surface marker CD27 to differentiate naïve from antigen experienced B cells. CD27 is a disulphide linked homodimeric glycoprotein involved in T cell activation and B cell regulation that is expressed by memory B cells, but not by naïve cells (1, 29, 30), and is considered the most reliable backbone marker for delineating memory status of B cells (29). In addition, CD27 can be used in combination with other markers to identify distinct B cell subpopulations and a number of different approaches have been proposed to fulfil this purpose (1, 29). In this investigation, we used two common approaches to differentiate immature B cells from naïve B cells: analysis of CD10 expression (5, 41), and analysis of IgD expression (25, 35). In addition, we used CD38 to differentiate plasmablasts / plasma cells from memory cells (5). We also investigated exercise-induced mobilisation of a recently purported 'B1' cell population, identified as CD27+CD43+CD69- (21). B1 cells represent a large B cell population whose primary characteristic is the production of low-affinity natural antibody that is broadly avid against invading pathogens; an exercise-induced mobilisation of these cells might be a beneficial adaptation that helps ward off infections during times of stress or physiological strain, as part of the "fight or flight" response. In the present study, we hypothesised that acute exercise would result in a greater mobilisation of effector B cells (i.e., memory and B1 cells) compared to naïve and immature B cells (8).

2. MATERIAL AND METHODS

2.1 Participants

Data for this investigation was collected from two independent exercise studies: study one was conducted in the School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, UK and study two was conducted in the Department of Health and Human Performance, University of Houston, Texas, USA.

In study one, peripheral blood mononuclear cells (PBMCs) were collected from nine participants (age: 22.1 ± 3.4 years; height: 180.5 ± 6.1 cm; weight: 78.1 ± 11.0 kg; body mass index: 24.0 ± 3.1 kg.m²; $\dot{V}O_{2\text{MAX}}$: 43.8 ± 4.1 ml.kg.min⁻¹) as previously described (44-46). All participants were non-smokers, and refrained from taking vitamin supplements and anti-inflammatory medication for fourteen days, and did not exercise and consume alcohol or caffeine for two days prior to testing. Participants also fasted overnight prior to the main exercise trial. All participants provided written consent and the study was approved by the Science, Technology, Engineering and Mathematics Ethical Review Committee at University of Birmingham.

In study two, PBMCs were collected from seven healthy males (age: 29.9 ± 4.7 years; height: 175.4 ± 10.9 cm; weight: 70.0 ± 7.9 kg; body mass index: 22.8 ± 1.8 kg.m²; $\dot{V}O_{2+5\% \text{ LT}}$: 47.6 ± 10.1 ml.kg.min⁻¹) as previously described (27). All participants were experienced cyclists, non-smokers, not taking medication or supplements affecting the immune system and were free from any infectious illness for 6-weeks prior to testing. Participants were asked to fast overnight and refrain from exercise 24 hours prior to testing. After receiving oral and written information pertaining to the risks and demands of the study, each participant signed an informed consent document and the study was approved by the committee for the protection of human subjects at the University of Houston.

2.2 Preliminary measurements

For both studies, height and weight were assessed using standard methods. For study one, cardiorespiratory fitness ($\dot{V}O_{2\text{MAX}}$) was measured using an incremental test to exhaustion on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). A facemask was fitted throughout the exercise test, and expired air was assessed for oxygen uptake and carbon dioxide production, averaged every 20 seconds, from breath-by-breath samples (*Oxycon Pro*,

Jaeger, Wuertzberg, Germany). Heart rate (HR) was monitored every minute using a Polar Vantage heart rate monitor (Polar Vantage, Kempele, Finland); maximal heart rate represents the maximum heart rate achieved during the trial. Following a three-minute warm up at 30 watts, workload was increased by 30 watts every minute, until volitional exhaustion. The following criteria were used to indicate that $\dot{V}O_{2\text{MAX}}$ had been reached: a fall in cadence below 60 rpm, a respiratory exchange ratio ($\dot{V}CO_2/\dot{V}O_2$) >1.10-1.15, plateau in participant oxygen consumption or a maximal heart rate >220 beats min^{-1} – age.

For study two, participants attended the laboratory to conduct an initial incremental discontinuous cycling test to determine individual blood lactate threshold (BLT). The initial BLT and subsequent exercise trial were conducted on participants' personal road bicycles mounted to an indoor cycle ergometer (Computrainer Lab, Racermate, Inc., Seattle, WA). Prior to the BLT, resting capillary blood samples were collected from the earlobe to determine resting blood lactate concentration. All blood lactate concentrations were determined immediately in duplicate using an automated pre-calibrated analyser (P-GM7 MicroStat, Analox Instruments, London, UK). After a 10 min warm-up consisting of cycling against no resistance, participants began the protocol which consisted of 3 min incremental stages. Initial starting workload increments were dependent on participant fitness levels and ranged from 50 – 100 W and increments of 20 – 25 W. Participants were instructed to maintain steady, comfortable revolutions per minute (RPM) throughout the test. Heart rate and breath-by-breath metabolic data ($\dot{V}CO_2$, $\dot{V}O_2$, ventilation and respiratory exchange ratio) were measured continuously using a metabolic cart (Quark CPET, Cosmed, Rome, Italy). The test was terminated one incremental stage after attaining a blood lactate concentration of >4 mM. The power output corresponding to the BLT was determined individually using the breakpoint method defined by Weltman (1995). In brief, blood lactate concentration (mM) was plotted against power (watts), with the threshold being determined as the power output corresponding to the break-point where a curvilinear rise in blood lactate concentration is first observed (48). For both studies, a rating of perceived exertion (RPE) was recorded using the Borg scale at each stage.

2.3 Experimental trials

For both studies, experimental trials were undertaken at least two days after the preliminary tests and were scheduled at a similar time of day. Prior to the exercise trial in study one, participants completed a warm up (5 min) at a workload eliciting 40% $\dot{V}O_{2\text{MAX}}$, before undertaking the steady state cycling task at 80% $\dot{V}O_{2\text{MAX}}$ for 20 minutes. A facemask was fitted throughout exercise for breath-by-breath measurements. Workload was expressed as watts, and relative to body mass (i.e., watts/kg). Values presented are the average workload over the entire exercise protocol and were obtained directly from the electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). Ratings of perceived exertion (RPE) were recorded using the Borg Scale every minute and RPE results are presented as the maximal RPE during the trial (44-46). In study two, participants completed a 30 min exercise trial at a workload corresponding to +5% of the individual blood lactate threshold. Following a 10 min warm-up, participants were instructed to cycle at the same cadence maintained during the BLT test; RPM was monitored and recorded every 5 min. Capillary blood samples were collected from the earlobe every 10 min for the analysis of blood lactate. In both studies, heart rate was measured every minute, and data were expressed as a percentage of age-predicted maximum heart rate using average heart rate and the equation $191.5 - 0.007 \times \text{age}^2$ (18).

2.4 Blood sampling, cell isolation and flow cytometry

For both studies, blood samples were drawn into potassium ethylene diaminetetraacetic acid (EDTA) vacutainer tubes (Becton & Dickson, Oxford, UK) after thirty minutes of rest, during the final minute of exercise, and thirty minutes later (study one) or 60 minutes later (study two).

In study one, blood from each time point was diluted 1:1 with Roswell Park Memorial Institute media (RPMI), and layered on top of Ficoll paque PLUS (GE Healthcare) (2:1), before centrifuging at $500 \times g$ for 30 minutes at 21°C . PBMCs were aspirated and washed three times in RPMI by centrifuging at $400 \times g$ for 5 minutes. The cell pellet was re-suspended in 1-millilitre of freezing mixture (70% RPMI, 20% fetal calf serum (FCS) and 10% dimethyl sulfoxide (DMSO)) and frozen at $-1^\circ\text{C}/\text{min}$ using a freezing container (Nalgene “Mr Frosty” ThermoScientific). Cells were

stored at -80°C and analysed within six months. For analysis, PBMCs were thawed rapidly at 37°C and washed twice in phosphate buffered saline (PBS) containing 2% FCS and 2mM EDTA (MACS buffer) by centrifuging at $400 \times g$ for 5 minutes. Cells were counted using a haemocytometer and approximately 300,000 PBMCs were added to tubes for incubation with fluorescently conjugated antibodies. Specific lymphocyte populations were identified by immunofluorescent monoclonal antibody (mAb) staining of whole blood by eight-colour flow cytometry (FACS-CANTO II, Becton–Dickinson, San Jose, USA). The following mAbs were used to stain cells using vendor-recommended concentrations: anti-CD3-V450 clone #UCHT1, anti-CD19-V500 clone #HIB19 (BD Horizon, San Diego, USA), anti-CD27-APC clone #M-T271, anti-CD43-FITC clone #IG10, anti-CD69-PE clone #FN50 (BD Pharmingen, San Diego, USA), anti-CD10-APC-Cy7 clone #HI10a, and anti-CD38-PE-Cy7 clone #HIT2 (BioLegend, San Diego, USA). Using these mAbs, CD3–CD19+ B cell subsets were classified into immature (CD27–CD10+), naïve (CD27–CD10–), memory (CD27+CD38–), plasma cells / plasmablasts (CD27+CD38+) (5), as well as recently purported ‘B1’ cells (CD27+CD43+CD69–) (21) (Table 1). 7-aminoactinomycin D (7-AAD; PerCP channel; BD Pharmingen, San Diego, USA) was used to exclude apoptotic and necrotic cells. FSC-area versus FSC-height was used for doublet discrimination. Flow cytometry analyses were conducted using FlowJo version 7.6.5 (FlowJo LLC, Oregon, USA). The total leukocyte count was assessed using an automated haematology analyser (Coulter Analyser, Beckman-Coulter, High Wycombe, UK).

In study two, PBMCs were separated from whole blood using almost identical methodology to study one, except blood was diluted and cells were washed in PBS. In addition, approximately 1,000,000 fresh PBMCs were stained in MACS buffer with the following fluorescently labelled mAbs using vendor-recommended concentrations: anti-CD20-PerCP clone #L27 (BD Biosciences, San Diego CA USA), anti-IgD-PE clone #IA6-2, Anti-IgM-PE clone #G20-127 (BD Pharmingen, San Diego CA USA), anti-IgA1-PE clone #B3506B4, anti-IgG-PE clone #H2 (Southern Biotech, Birmingham AL USA) and anti-CD27-FITC clone #0323 (eBioscience, San Diego CA USA). Using these mAbs, CD20+ B cells were classified into immature (CD27–IgD–), naïve / transitional (CD27–IgD+), and IgG+/IgA+ class switched memory cells (CD27+IgD–) (30, 40) (Table 1). Cellular analyses were conducted on a BD Accuri C6 (Becton Dickinson San Jose CA USA) flow

cytometer, as previously described (42). Flow cytometry data analyses were conducted on CFlow[®] Software (CFlow[®] software V2). Total leukocyte count was quantified on a flow cytometer by mixing whole blood with a red cell lysing buffer (eBioscience, San Diego CA USA) for 15 min and the number of gated lymphocytes counted in 40µl of lysed blood at a flow rate of 14µl/min was determined and then adjusted by the dilution factor. This flow cytometry-based technique was internally validated using a clinical haematology analyser (BC3200 Mindray North America, Mahwah NJ USA).

To ensure consistency between laboratories for study one and study two, all antibody panels were first piloted in Birmingham. For validation purposes, fluorescence-minus-one (FMO) tubes were used on separate samples from three healthy donors to establish negative and positive gating strategies for all B cell surface markers.

2.5 Statistical analyses

All results are presented as mean \pm standard deviation unless otherwise stated. Statistical calculations were performed on IBM SPSS for Windows Version 21. Kolmogorov-Smirnov tests were used to test for normally distributed data. Repeated Measures Analysis of Variance (ANOVA) was used to contrast changes over time (baseline, exercise and +30 min post-exercise for study one or +60 min post exercise for study two), with between group factors used to assess differences between study one and two, where necessary. Post-hoc pairwise comparisons between time-points were performed using Bonferroni adjustment for multiple comparisons. Between study differences at a given time point (physiological data in Table 2) were assessed using one-way ANOVA. Statistical significance was accepted at the $p < .05$ level.

3. RESULTS

3.1 Physiological responses to exercise and workload measurements

All participants completed the exercise protocol in each study. Table 2 displays the summary data for power output, workload, maximal heart rate during exercise, and maximal rating of perceived exertion. As expected, a significant increase in all of these variables ($p < .01$) was observed during both exercise trials. Given that the trials were not intensity-matched, the trials differed significantly in terms of power output and workload. As outlined in Table 2, participants in study one reported a higher rating of perceived exertion compared to participants in study two, whereas participants in study two exhibited a higher average power output and workload compared to participants in study one. Participants in study two had a higher $\dot{V}O_{2\text{MAX}}$ compared with those taking part in study one. There were no other significant differences between the participants taking part in each study, or for any other baseline parameters measured.

3.2 Effects of acute exercise on total lymphocytes and total B cells

Table 3 displays total lymphocyte numbers before, during and after each of the exercise trials. As expected, total lymphocyte counts increased significantly during both exercise trials ($p < .01$), and we observed a greater increase in total lymphocytes from baseline to the final minute of exercise for study one (+150%) compared to study two (+80%) ($F_{(2,14)} = 13.1$; $p = .003$). In the recovery period post-exercise, there were no significant decreases in total lymphocyte number at 30 minutes (study one) or 60 minutes (study two) post-exercise compared to pre-exercise values ($p > 0.073$).

The effects of each exercise trial on total B cell numbers is displayed in Table 3. In both studies, B cells increased from baseline to the final minute of exercise. Despite differences in exercise intensity, we found no differences between studies for the magnitude of total B cell mobilisation ($p = \text{NS}$). CD19+ B cells returned to resting levels at 30 minutes post-exercise in study one. A marginal decrease in CD20+ B cells below resting levels was observed at 60 minutes post-exercise in study two.

suggesting that B cells may leave the bloodstream to peripheral sites post-exercise, as previously reported (8).

3.3 Effects of acute exercise on B cell subsets

Table 3 shows the effects of each exercise trial on B cell subsets. All B cell subsets increased significantly during exercise in study one ($p < .01$). The largest numerical contributors to the increase in CD19+ B cells were naïve cells. However, there was an overall trend whereby immature B cells showed a greater proportional increase than naïve and memory B cells (see Figure 1); though, these differences were not significant. Immature, naïve and memory B cells returned to resting levels at 30 minutes post-exercise. Plasmablasts and purported 'B1' cells also increased during exercise, and returned to resting levels at 30 min post-exercise (Table 3). Unlike study one, we did not observe a significant increase across all B cell subsets during exercise in study two. Indeed, main effects of time were only observed for immature and total memory B cells ($p < .01$); these cells returned to baseline levels at 60 minutes post-exercise. No significant increases were observed for class-switched IgA+ or IgG+ memory cells ($p = \text{NS}$). Like study one, immature B cells appeared to be more sensitive to exercise than memory and naïve B cells (Figure 1).

To further elucidate whether immature B cells were more responsive to exercise than other B cell phenotypes in each study, we compared the percentage change in immature cells with the percentage change in all other B cell subsets, but no differences were observed ($p = \text{NS}$; data not illustrated). However, upon combining data from both studies, an effect was found whereby immature cells were mobilised more than other B cell subsets (see Figure 2), giving an indication that exercise may preferentially increase the number of immature cells over other B cell subsets ($F_{(1,30)} = 4.9$; $p = .034$). This approach is justified because cell surface CD10+ and IgD- expression on CD27- B cells overlaps and broadly represents immature B cells (5, 30, 40). Using the same approach of combining other data from the two exercise studies did not yield significant results for other B cell subsets, further supporting the finding that immature B cells are most responsive to exercise stress.

4. DISCUSSION

This is the first study to document the effects of acute exercise on the mobilisation of a broad range of phenotypically distinct B cell subsets. We found, in the two independent exercise trials reported in this study, that immature B cells showed larger mobilisation responses than memory and naïve B cells.

To date, the phenotypic composition of B cell mobilisation during exercise has been poorly characterised. This is despite numerous findings showing that total B cells increase two-fold during strenuous cycling (4), intensive running (14), maximal rowing exercise (36), and after strenuous resistance exercise (31, 32). A similar effect is observed during psychological stress (8). In the aforementioned stress study it was shown that CD62L⁺ B cells are preferentially mobilised, and, as CD62L is not expressed by antigen-experienced, skin-homing B cells (17, 49), it was hypothesised that this finding represented the first step in the redirection of antigen-experienced B cells to peripheral tissues. This is partly supported by our results where memory B cells appeared to increase more than naïve B cells, albeit marginally. Despite these findings, the apparent larger mobilisation of immature B cells observed in our study appears to contradict the hypothesis that effector B cells (i.e., with an antigen-experienced phenotype) are preferentially mobilised by stress or exercise. Importantly, it has been shown that repeated exposure to acute stressors appears to redirect B cells from the bone marrow to the spleen (13). It is suggested that the primary reason B cells traffic to the spleen (i.e., the largest secondary lymphoid organ) after stress is to prepare for body injury and subsequent pathogenic infection so that large numbers of naïve B cells are ‘on-site’ to efficiently screen for antigen targets (13). The findings of our study may support this standing given that immature cells, which have yet to interact with cognate antigen, were preferentially increased in the bloodstream for presumed later redistribution to secondary lymphoid organs, where subsequent maturation and antigenic screening occurs.

In order to phenotype circulating B cells, each of the studies in this investigation used CD27 as a backbone marker to distinguish between naïve and memory cells (29). It is clear however, that

phenotyping memory B cells is not straightforward, and these cells exhibit many levels of plasticity, and are likely comprised of a range of memory subsets that may include T cell-dependent, T cell-independent, class-switched and class-unswitched phenotypes, among others (28, 40). For this reason, further markers – in addition to CD27 – are needed to more precisely characterise B cell subset responses to exercise. The challenges associated with B cell phenotyping in humans is highlighted in the context of a population of purported ‘B1’ cells (21), which were also measured in the study herein. ‘B1’ cells are a population of B cells, originally identified in mice that produce high levels of low-affinity natural antibody. In rats, it has been shown that natural IgM and B1 cells are increased in physically active versus sedentary animals, but the effects of exercise in humans has remained unclear (11, 12). Recently, however, it has been shown that the phenotypic markers proposed for purported human ‘B1’ cells are, in fact, a population of pre-plasmablasts in humans (6). In agreement with the latter paper, we found that approximately 50% of ‘B1’ cells also expressed CD38 (data not shown) and thus likely represented a plasmablast phenotype (6). Finally, although the two exercise protocols in this investigation led to significant changes in the percentages of circulating immature B-cells, it is important to acknowledge that the absolute numbers of cells mobilised into peripheral blood remained modest. Future studies may be able to provide insights on the biological significance of the exercise-induced changes in the immature B cell compartment.

A limitation of our study is that the intensity of exercise, or perhaps more substantially, the duration of exercise, may have been insufficient to stimulate a large magnitude change in B cell numbers during and following each trial. This idea is partly supported by the fact that study one – which resulted in a higher RPE and heart rate than study two – mobilised total B cells to a greater extent. A dose-response relationship between exercise intensity and the magnitude of lymphocytosis is well known (4, 22). It has also been established that the magnitude of exercise-induced cortisol and catecholamine release, which have been implicated in maintaining lymphocytopenia (9, 2), are strongly influenced by exercise intensity and duration (15, 16, 22, 24). In the work presented herein, in the recovery period following exercise (30 minutes in study one, and 60 minutes in study two) we did not observe a B cell lymphocytopenia. Thus, it is likely that an exercise stimulus greater than the

20-30 minutes examined in the present work would result in a more substantial post-exercise egress of cells from the bloodstream (15, 16, 22). Therefore, via the implementation of more strenuous and prolonged exercise protocols, with a larger number of participants, future studies may be able to better elucidate the phenotype of B cell subsets mobilised by exercise and those leaving the bloodstream in the post-exercise period. A final consideration is that although it was our intention to employ different exercise protocols and different cell phenotyping strategies between the two studies, study one undertook flow cytometry analysis on PBMCs that had been cryopreserved, whereas study two undertook analyses on fresh PBMCs. There is conflicting evidence to suggest that freezing and thawing cells can lead to a selective loss of certain peripheral blood cell sub-types (19, 38). However, in this work we generally observed similar exercise-induced patterns in B cell mobilisation between the two exercise studies. In addition, the viability of our cryopreserved cells was >98% in all samples as shown by 7AAD exclusion (data not shown). Thus, it is unlikely that cryopreservation affected the overall results.

5. CONCLUSION

This investigation found, by employing two separate exercise protocols, and by using complimentary phenotyping strategies, that exercise results in a mobilisation of B cells, of which the largest numerical contributors were naïve cells. As expected, very few plasma cells and class switched IgG+ / IgA+ memory cells were present in the peripheral blood, and did not appear to change substantially during exercise. Immature B cells showed that largest magnitude of change in response to exercise, in both exercise trials, followed by memory B cells and naïve cells. Thus, our findings show that, unlike T cells and NK cells (3, 4), B cell mobilisation is not driven by effector status, and, for the first time, that B cell mobilisation during exercise is partly comprised of immature CD27–IgD–/CD10+ cells, which may aid in the screening of novel pathogens.

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ACCEPTED MANUSCRIPT

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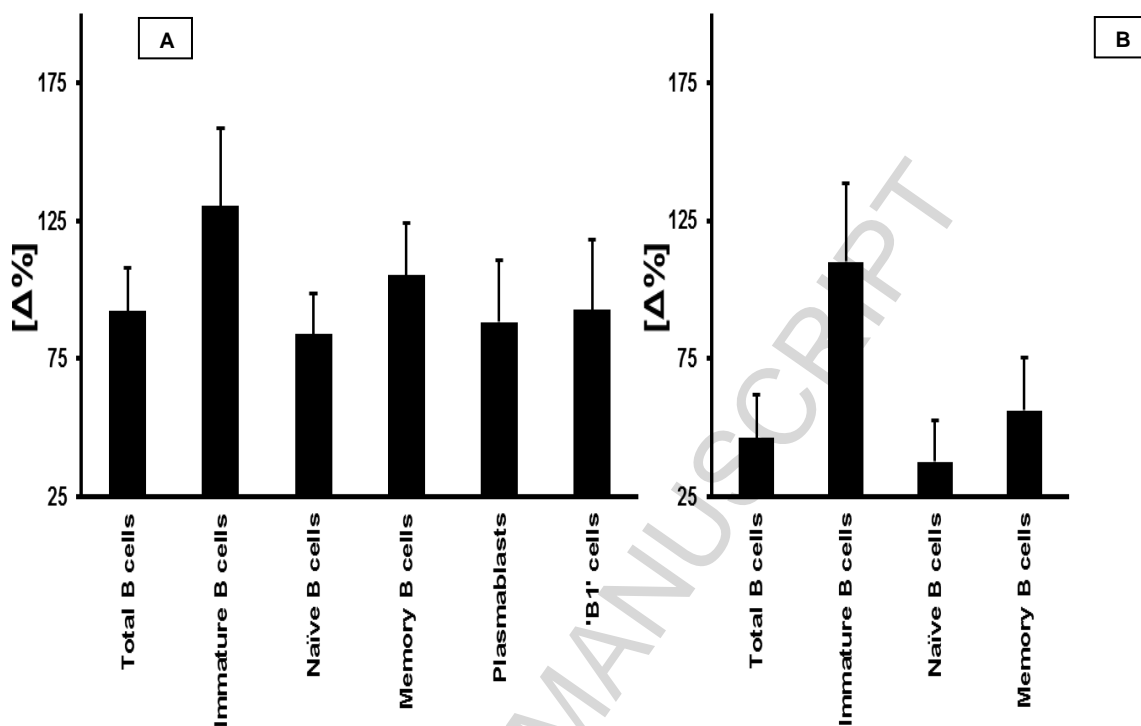
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FIGURE CAPTIONS

Figure 1. Mean \pm SEM percentage changes to B cell subset numbers, from baseline to immediately post-exercise, in study one (A) and study two (B). Within each trial, no significant differences were observed between each subset.

Figure 2. Mean \pm SEM percentage changes from baseline to immediately post-exercise of immature B cell subsets compared to all other B cells, from both exercise trials combined. * indicates significant difference ($p < 0.05$) observed between B cell subsets.

**Fig. 1**

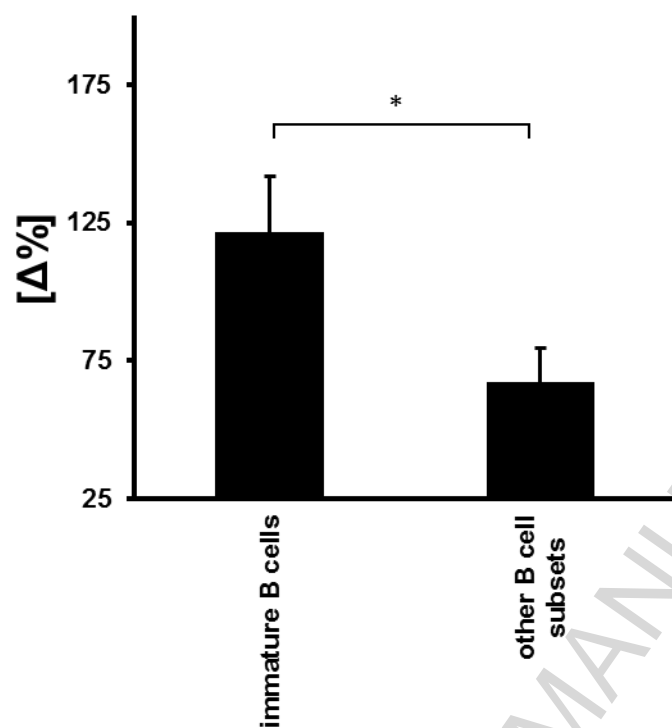


Fig. 2

Table 1. B cell subset phenotypes characterised in study one and study two.

| B cell subset name | Phenotype |
|-----------------------------|--------------------------|
| Study one | |
| B cells | CD3–CD19+ |
| Immature B cells | CD3–CD19+CD27–CD10+ |
| Naïve B cells | CD3–CD19+CD27–CD10– |
| Memory B cells | CD3–CD19+CD27+CD38– |
| Plasmablasts / plasma cells | CD3–CD19+CD27+CD38+ |
| ‘B1’ cells | CD3–CD19+CD27+CD43+CD69– |
| Study two | |
| B cells | CD20+ |
| Immature B cells | CD20+CD27–IgD– |
| Naïve B cells | CD20+CD27–IgD+ |
| Memory B cells | CD20+CD27+IgD– |
| IgA+ memory B cells | CD20+CD27+IgA+ |
| IgG+ memory B cells | CD20+CD27+IgG+ |
| IgM+ memory B cells | CD20+CD27+IgM+ |

Table 2. Exercise physiology data collected from participants enrolled in study one and study two.

| | Study 1 | Study 2 |
|---|-----------------|----------------|
| Average power output (Watts) | 170.30 ± 33.27* | 202.00 ± 62.00 |
| Average workload (Watts/kg) | 2.18 ± 0.33* | 2.87 ± 0.81 |
| Maximal heart rate (bpm) | 187 ± 7 | 157 ± 11 |
| Maximal heart rate (%) ^a | 94 ± 4 | 85 ± 6 |
| Rating of perceived exertion (Borg Scale) | 18 ± 1 * | 14 ± 1 |

* indicates significant difference between study one and study two ($p < .05$)

^a calculated as a percentage of age predicted maximum using the equation: $191.5 - 0.007 \times \text{age}^2$ (Gellish et al., 2007).

Table 3. Changes in the numbers (cells per μL) of B lymphocyte lineage cells in response to high intensity exercise during study one (A) and study two (B).

| A) Study one | | | | | |
|-------------------------------|-----------------------------|--------------------|-----------------------|--------------------|---------------------------------|
| Cell type | Phenotypic characterisation | Baseline | Exercise | +30min | Main effects of time |
| Lymphocytes | | 1911.1 \pm 410.6 | 4833.3 \pm 1330.4** | 1844.4 \pm 353.9 | $F_{(2,16)} = 53.310; p < .001$ |
| B cells | CD3-CD19+ | 161.8 \pm 69.1 | 304.2 \pm 127.2** | 127.6 \pm 38.4 | $F_{(2,16)} = 19.332; p = .001$ |
| Immature B cells ‡ | CD3-CD19+CD27-CD10+ | 14.1 \pm 8.7 | 28.2 \pm 12.8** | 11.5 \pm 5.2 | $F_{(2,16)} = 21.615; p < .000$ |
| Naïve B cells ‡ | CD3-CD19+CD27-CD10- | 98.5 \pm 47.2 | 177.7 \pm 80.4** | 73.6 \pm 27.1 | $F_{(2,16)} = 17.923; p = .001$ |
| Memory B cells ‡ | CD3-CD19+CD27+CD38- | 47.3 \pm 23.1 | 95.2 \pm 55.4* | 41.0 \pm 16.2 | $F_{(2,16)} = 11.967; p = .007$ |
| Plasmablasts / plasma cells ‡ | CD3-CD19+CD27+CD38+ | 2.0 \pm 1.9 | 3.2 \pm 2.2* | 1.5 \pm 1.0 | $F_{(2,16)} = 10.465; p = .003$ |
| 'B1' cells † | CD3-CD19+CD27+CD43+CD69- | 4.3 \pm 2.0 | 7.9 \pm 3.4* | 3.7 \pm 1.7 | $F_{(2,16)} = 13.805; p = .002$ |

‡ estimated using the method of Caraux et al., 2010

† estimated using the method of Griffin et al., 2012

| B) Study two | | | | | |
|---------------------|-----------------------------|--------------------|-----------------------|--------------------|---------------------------------------|
| Cell type | Phenotypic characterisation | Baseline | Exercise | +60min | Main effects of time |
| Lymphocytes | | 1494.7 \pm 643.4 | 2697.1 \pm 1104.7** | 1201.3 \pm 473.6 | $F_{(2,12)} = 19.78; p = .004$ |
| B cells | CD20+ | 139.0 \pm 86.3 | 220.9 \pm 129.4* | 120.8 \pm 90.7* | $F_{(2,12)} = 14.567; p = .004$ |
| Immature B cells | CD20+CD27-IgD- | 7.6 \pm 6.35 | 15.3 \pm 11.6 | 5.9 \pm 3.1 | $F_{(2,10)} = 5.19; p = .044$ |
| Naïve B cells | CD20+CD27-IgD+ | 89.6 \pm 74.6 | 130.1 \pm 116.2 | 84.1 \pm 68.6 | $F_{(2,10)} = 4.84; p = .069$ [NS] |
| Memory B cells | CD20+CD27-IgD- | 18.7 \pm 11.2 | 29.0 \pm 15.9 | 17.3 \pm 12.9 | $F_{(2,12)} = 6.23; p = .023$ |
| IgA+ memory B cells | CD20+CD27+IgA+ | 1.8 \pm 1.2 | 2.6 \pm 2.5 | 1.9 \pm 2.2 | $F_{(2,12)} = 0.073; p = \text{NS}$ |
| IgG+ memory B cells | CD20+CD27+IgG+ | 7.6 \pm 5.6 | 11.6 \pm 10.7 | 6.0 \pm 5.4 | $F_{(2,12)} = 0.073; p = \text{NS}$ |
| IgM+ memory B cells | CD20+CD27+IgM+ | 0.6 \pm 0.4 | 0.7 \pm 0.8 | 0.4 \pm 0.3 | $F_{(2,12)} = 1.814; p = \text{NS}$ |

* $p < .05$ in comparison to baseline

** $p < .01$ in comparison to baseline

NS $p > .05$

Highlights

- We assessed the effects of acute exercise on B lymphocyte subset mobilisation
- Immature B cells showed the largest magnitude increases during exercise
- Naïve B cells were the largest numerical contributors to the exercise lymphocytosis
- Few plasma cells / plasmablasts were present in blood before and after exercise